

Total fluoride intake and urinary excretion in 4-year-old Iranian children residing in low-fluoride areas

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Knowledge of levels of fluoride ingestion and excretion is important in planning optimum fluoride therapy for young children. In previous literature, it has been assumed that only about one-third of ingested fluoride is excreted in young children. The aims of the present study were (a) to measure total fluoride intake, urinary fluoride excretion and fluoride balance, and (b) to investigate the effect of air temperature on fluoride intake and urinary fluoride excretion, in young children. Children (4 years old) living in a city, a small town and rural areas of Fars province, Iran, where drinking water contained 0.30–0.39 mg F/l, were invited to participate. Selection of subjects was by random sampling of kindergartens or health centres. The children were surveyed twice, once in summer and once in winter. Diet was obtained by 3 d diaries with interview. Samples of most foods and drinks were analysed for fluoride content. Ingestion of fluoride from toothpaste was estimated for each child. Each child's urine was collected over 24 h and analysed for fluoride content. Seventy-eight of the 116 volunteers completed all aspects of the study, which was conducted in 1995–6. For all children, the mean fluoride ingestion from diet was 0.390 (SD 0.422) mg/d or 0.028 (SD 0.008) mg/kg body weight per d. Fluoride ingestion from diet was higher in summer and higher in rural areas. The mean ingestion of fluoride from all sources was 0.426 (SD 0.426) mg/d and the mean fluoride urinary excretion was 0.339 (SD 0.400) mg/d. The difference between ingestion and urinary excretion was +0.087 (SD 0.443) mg, equivalent to 80 % excretion. Faecal excretion was not estimated. The results indicate fluoride retention at 4 years to be much lower than previously assumed.

Fluoride: Children

Food, including water, toothpaste and other caries preventive agents such as tablets, are the main contributors to fluoride intake. The more widespread use of fluorides is likely to have increased fluoride ingestion and recent studies in the USA have shown increases in the prevalence of enamel fluorosis in non-fluoridated and fluoridated areas (Pendrys & Stamm, 1990). This trend has also been reported in Europe (Woltgens *et al.* 1989) and New Zealand (Cutress *et al.* 1985). In an attempt to advise on fluoride intake, ingestion of 0.05 to 0.07 mg/kg body weight per d has been considered as 'optimal' during mineralization of the teeth, for greatest resistance to dental caries and freedom from dental fluorosis (Levy *et al.* 1995). Mild mottling may begin to occur when daily fluoride intake is 0.1 mg/kg body weight per d during this period of mineralization, which is between birth and about 5 years for the aesthetically important incisor teeth. However, such recommendations are based on limited data, as there have been few studies where total fluoride intake (from diet, including

water, dentifrice and dietary supplements) in children, has been measured.

Dietary fluoride intake for infants and children younger than 2 years old in fluoridated and non-fluoridated areas has been reported in several studies. The daily amounts of dietary fluoride intake have ranged from 0.23 to 0.76 mg in fluoridated areas (Singer & Ophaug, 1979; Dabeka *et al.* 1982; Ophaug *et al.* 1985; Featherstone & Shields, 1988; Hattab & Wei, 1988; Guha-Chowdhury *et al.* 1990) and from 0.08 to 1.23 mg in non-fluoridated areas (Wiatrowski *et al.* 1975; Singer & Ophaug, 1979; Dabeka *et al.* 1982; Ophaug *et al.* 1985; Featherstone & Shields, 1988; Guha-Chowdhury *et al.* 1990). However, there have been fewer studies which have determined daily fluoride intake from diet for 3–6-year-old children residing in fluoridated areas (Schamschula *et al.* 1988b; Burt, 1992; Pang *et al.* 1992; Guha-Chowdhury *et al.* 1996), a range from 0.36 to 0.90 mg, while there have been very few studies on the dietary fluoride intake for 3–6-year-olds living in non-fluoridated

Abbreviation: PAL, physical activity level.

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areas: a mean of 0.22 mg/d was reported by Schamschula *et al.* (1988b), and 0.45 mg/d by Guha-Chowdhury *et al.* (1996). In none of these studies was the effect of temperature on water intake, and consequently on fluoride intake, considered: this is a relevant factor in many countries.

In many communities, fluoride dentifrices are likely to have made an important contribution to the total fluoride intake in young children. Several studies have revealed that 28–49 % of dentifrice used is ingested by children aged 3–6 years and the mean amount of fluoride ingested per brushing episode is reported to range from 0.8 to 0.44 mg (Ericsson & Forsman, 1969; Hargreaves *et al.* 1972; Simard *et al.* 1989; Naccache *et al.* 1990, 1992; Guha-Chowdhury *et al.* 1996). Dietary fluoride supplements have been recommended for children at risk of dental caries living in areas receiving fluoride-deficient water supplies.

There is only one report of total fluoride intake from all the previously mentioned sources in young children. In this recent study, which involved the collection of duplicate portions of all foods and drinks consumed by sixty-six 3–4-year-old children living in low-fluoride areas (0.2–0.3 mg F/l water) of New Zealand, the total fluoride intake, from diet and toothpaste but excluding dietary fluoride supplements, ranged from 0.7 to 1.21 mg/d, with a mean of 0.49 mg/d (or 0.027 mg/kg body weight per d) (Guha-Chowdhury *et al.* 1996). Other available data on the total fluoride intake from all sources of fluoride in young children are based on estimations from different studies and not based on the measurement of actual intake by a group of children.

Urinary analysis has long been recognized as the major variable for assessing ingestion of fluoride by assuming that about 50 % of ingested fluoride would have been excreted in urine in adults and about 30 % in children (World Health Organization, 1986). There are several studies on the concentration of fluoride in spot samples of urine from children while only in a few studies have the total volume and weight of fluoride excreted in 24 h urine samples been measured (Crosby & Shepherd, 1957; Rugg-Gunn *et al.* 1993; Warpeha & Marthaler, 1995), partly due to the difficulties of obtaining 24 h urine collections, especially in young children.

The number of studies on fluoride balance is limited, especially for young children, and data are conflicting. Ekstrand *et al.* (1984) found that breast-fed infants, with daily fluoride intakes ranging from 5 to 19 g, were in negative fluoride balance, whereas the fluoride balance was positive for formula-fed infants who received daily fluoride intakes ranging from 891 to 1012 g. The only report of

fluoride balance for children aged 3–4 years is an abstract by Brunetti & Newbrun (1983). They reported a very low fluoride balance of +0.05 mg F/d (15 % of ingested fluoride retained). This 85 % excretion of ingested fluoride was very much higher than the up to 30 % assumed by World Health Organization (1986). There is a need for further information on this issue.

The aims of the present study were (a) to determine fluoride intake, urinary fluoride excretion and balance, and (b) to investigate the effect of climate temperature on fluoride intake and urinary fluoride excretion, in 4-year-old children.

Materials and methods

Preliminary investigation

The study was undertaken in Fars Province, south-west Iran. As there was no recent and reliable information on the fluoride concentration in drinking water in Iran, water samples were collected from twelve sites in Fars province in a preliminary study. These were obtained from the water tap that people used for drinking and cooking, at three different times of the day. The fluoride content of each water sample was determined using a fluoride-ion-selective electrode (model 96–09, meter model 720A; Orion, MA, USA) after adding one part of total ionic strength adjusted buffer III (Orion Research Inc., Cambridge, MA, USA) for every ten parts of each sample. The results revealed a range of mean concentrations for these sites of 0.6–4.05 mg F/l.

As a result of these preliminary studies, the main study was undertaken in six localities, but the results reported in the present paper refer only to the four localities receiving water containing 0.30–0.39 mg F/l (Table 1). The remaining two localities had mean water fluoride concentrations of 0.56 and 4.05 mg/l. Three types of locality were selected: an area with predominantly high-socio-economic status inhabitants in a big city (Shiraz; population 1.8 million); Darab, a small town (population 0.2 million) 300 km south of Shiraz; and two rural areas (Table 1) situated 10–30 km from Darab. The main study took place in May–July 1995 and December 1995–March 1996, with mean maximum day temperatures of 29° and 5° respectively.

Subjects

Consent for this study was obtained from the local Civic Welfare Organization in Iran. In total, 130 healthy children

Table 1. Fluoride concentration (mg/l) in the drinking water in four different Iranian localities, in summer and winter (Mean values and standard deviations)

Area	Summer			Winter			Average of both seasons		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Urban:									
Shiraz	0.32	0.10	9	0.31	0.10	25	0.32	0.10	34
Darab	0.38	0.05	7	0.32	0.05	15	0.35	0.05	22
Rural:									
Dehkhir	0.35	0.06	4	0.34	0.04	13	0.34	0.04	17
Hassan-Abad	0.39	0.08	8	0.22	0.06	10	0.30	0.10	18

aged 4 years from both sexes, residing since birth in areas with about 0.8 mg F/l in domestic water were selected, and informed parental permission for each child's participation was sought. The study population was recruited via kindergartens in the two urban areas and health centres in the two rural areas, by random sampling of these units. Parents who agreed to their child's participation were interviewed to obtain some general information about their children such as the child's toothbrushing habits and dietary habits in each season. The height and weight of each child were measured, the same stadiometer (± 2 mm) and spring scales (± 50 g) being used for all children.

Dietary assessment

Dietary information was collected by a 3 d dietary diary followed by a private interview on the fourth day (Hackett *et al.* 1983; Adamson *et al.* 1992), once in summer and once in winter. Diaries, which were pocket-size and robust, so as to be carried easily, were given to each parent with instructions on how to complete the dietary diary and the importance of recording all food, and especially drink, consumed over the 3 d period. The private interview on the day immediately after completing the 3 d diary was held to (a) ensure that all food and drink items were entered into the diary, (b) clarify the nature of the food or drink, and obtain the recipe of any home-made food and drink if necessary, and (c) determine the weights of food and drink consumed from household descriptions using calibrated measures and models (Adamson *et al.* 1992).

Measurement of fluoride intake from foods and drink

Several samples of almost all types of food and drink consumed by the children were collected, weighed and frozen until analysis. The acid-diffusible fluoride in the samples was isolated by the hexamethyldisiloxane diffusion method of Venkateswarlu (1992) with minor modification (Zohouri & Rugg-Gunn, 1999) and measured using a fluoride-ion-selective electrode. Of a total of 917 kg of different kinds of food and drinks consumed by the children, 800 kg (87%), including the main local staple foods (e.g. bread, cooked rice and cooked vegetables), was analysed for fluoride content. The fluoride concentration of the remaining 13 % of foods and drinks not collected for analysis but consumed by the subjects, was estimated using the most appropriate published data (Taves, 1983; Lopez & Navia, 1988; Schamschula *et al.* 1988a). These concentrations of fluoride ($\mu\text{g}/100$ g food) were added to computerized standard food tables (Holland *et al.* 1991). The dietary data were then analysed to determine the daily intake of nutrients, calculated for each child as the mean over 3 d (summer or winter) and 6 d (both seasons).

Estimation of validity of dietary information and analytical method

The validity of dietary information was estimated in two ways: first, by calculating the physical activity level (PAL) for the subjects and, second, by comparing the energy intakes of these subjects with those obtained in other

studies. BMR for 4-year-old children was calculated from body weight using standard equations determined by Schofield (1985) as follows:

$$\begin{aligned}\text{male BMR (MJ)} &= 0.095 (\text{weight (kg)}) + 2.410; \\ \text{female BMR (MJ)} &= 0.085 (\text{weight (kg)}) + 2.033.\end{aligned}$$

The PAL was calculated by dividing the mean recorded energy intake of these children by the calculated BMR. For most people in the UK, a PAL of 1.4 is applicable (Department of Health, 1991) implying moderate physical activity. A mean energy intake of less than $1.4 \times \text{BMR}$ for a group of adults almost certainly implies underestimation of dietary intake (Bingham, 1994). As the second method of determining validity, the mean energy intake of these children was compared with published data and reference values (Department of Health, 1991).

To evaluate the validity of the analytical method for fluoride analysis, 0.40, 0.20 and 0.50 μg fluoride were added to approximately half of the food samples before diffusion and the percentage fluoride recovery measured. These food samples were analysed alongside samples without the added fluoride. To measure the reliability of the method, fluoride measurement of the food samples was repeated in 10 % of samples.

Measurement of fluoride ingestion from toothpaste

Children with toothbrushing habits were asked to brush their teeth, based on their usual habits using their normal toothbrush and toothpaste. The brand of toothpaste used by the child was noted. Toothpaste was dispensed by parent or child onto the toothbrush, and the brushing was performed by the child with or without assistance; finally, the child would expectorate or rinse out their mouth if desired. The amount of toothpaste used was measured by weighing the child's toothbrush before and after dispensing the toothpaste. During toothbrushing and rinsing, all expectorated saliva, liquids and toothpaste were collected in a wide-mouth plastic container and any toothpaste adhering to the face or hands collected using a spatula and transferred to the container. The toothbrush and spatula were thoroughly rinsed with tap water into the container. After measuring the volume and thoroughly mixing the contents of the container, two samples of the mixture were put into polystyrene tubes and frozen at -20° . These procedures were undertaken once in summer and once in winter. Samples of the actual toothpaste used by each child were not collected for analysis of fluoride concentration, but samples of each toothpaste on sale in that region were purchased. Regardless of whether these purchased samples of toothpastes were labelled for fluoride content, all of them were analysed for fluoride concentration. The concentrations of fluoride in toothpastes and expectorated saliva were measured using a fluoride-ion-selective electrode after preparing a single aqueous extract of toothpaste or sample and separation of fluoride in sodium monofluorophosphate toothpastes by acid phosphatase (EC 3.1.3.2) (Duckworth *et al.* 1991). The amount of fluoride used per brushing episode (μg), for each child on each of two occasions was calculated by multiplying the weight of toothpaste used (g) by fluoride concentration in the toothpaste used by that child ($\mu\text{g}/\text{g}$).

The amount of fluoride expectorated (μg), for each child on each occasion, was calculated by multiplying the weight of expectorated liquid (assuming 1 ml = 1 g) by the concentration of fluoride in the expectorated liquid ($\mu\text{g/g}$) after subtracting the fluoride concentration of the tap water (as the children used tap water to rinse their mouth and wash the brush after use). Fluoride ingested per brushing (μg) was obtained by subtracting the amount of fluoride expectorated from the amount initially put on the toothbrush. Using the information on frequency of brushing, the amount of fluoride ingested per day was calculated for each child. The recovery of fluoride added as sodium monofluorophosphate to samples of expectorated liquid was measured to estimate the validity of the analytical procedure.

Measurement of fluoride excretion

A child's potty, funnel and a 1.5 litre plastic collection bottle with screw top, which contained 250 ml chlorhexidine digluconate (200 ml/l) as preservative, were given to each family. The parents were instructed how to collect their child's urine during 24 h: the importance of collecting all urine over the 24 h period was stressed. After collection, parents were questioned about the completeness of the collection and, if there was doubt, arrangements for a further 24 h collection were made. Urine volume was measured and two samples placed in 7 ml polystyrene tubes and frozen. The 24 h urine collections were also undertaken in summer and winter during the same period as the dietary assessment. The fluoride concentration of samples was measured by the fluoride-ion-selective electrode.

Estimation of the validity of 24 h urine volume

The validity of 24 h urine volume was estimated by measuring urinary creatinine by the Jaffé method (Bones & Taussky, 1951) using a commercial kit (Uni-Kit II; Roche Diagnostics, Welwyn Garden City, Herts., UK) and comparing these data with standards, using the formula proposed by Harms & Scharfe (1997): $\text{mg urinary creatinine/kg per d} = 15 + (0.5 \cdot \text{age year}) \pm 3$. Urinary creatinine for 4-year-old children ranges from 14 to 20 mg/kg per d.

Data analysis

Descriptive data were obtained. As no hypotheses were proposed, no analytical statistics have been determined.

Table 2. Distribution of subjects who volunteered and who participated in all parts of the present study, according to sex, in the four areas of Iran

(Values are numbers of subjects)

Area	Subjects who volunteered			Participants in all parts of study		
	Boys	Girls	Total	Boys	Girls	Total
Urban:						
Shiraz	22	19	41	17	15	32
Darab	20	22	42	12	16	28
Rural:						
Dehkhir	9	13	21	5	5	10
Hassan-Abad	6	6	12	4	4	8
Total	57	60	116	38	40	78

Results

Water samples

The fluoride concentrations of ninety-one samples of drinking water, twenty-eight samples collected in summer and sixty-three samples collected in winter, are given in Table 1. The overall mean fluoride concentration in all areas was 0.85 mg/l.

Fluoride analyses

The overall mean recovery of fluoride added to sixty food samples before diffusion, ranged from 83 to 105 %, with a mean of 98 (SD 5) %. Results of the duplicate analysis of twelve food samples were within ± 3 %. In all, 117 separate foods and drinks (excluding drinking water which was analysed in each area on a number of occasions (Table 1)) were analysed out of a total of 172 different food items consumed by the children. The mean fluoride concentration was below 1.0 $\mu\text{g/g}$ for most of them, and for thirty of them, below 0.4 $\mu\text{g/g}$. These 117 foods and drinks were the major components of the diet (800 kg food consumed out of a total of 917 kg) and contained 90 % of the fluoride in the diet, so that the remaining dietary items (whose fluoride concentration was obtained from published sources) contained only 10 % of dietary fluoride. The mean recovery of sodium monofluorophosphate added to all toothpaste samples was 96 (SD 5) %. Three of the toothpastes obtained contained no fluoride. All samples were analysed in duplicate. The mean total fluoride concentration (n 2) of the three commercially available Iranian children's toothpastes ranged from 348 to

Table 3. Fluoride intake (mg/d) from the diet during summer and winter in seventy-eight children from four different locations in Iran (Mean values and standard deviations)

Location . . .	Dietary fluoride intake (mg/d)							
	Shiraz (n 32)		Darab (n 28)		Dehkhir (n 10)		Hassan-Abad (n 8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Summer	0.838	0.085	0.437	0.108	0.607	0.176	0.545	0.201
Winter	0.299	0.097	0.290	0.092	0.544	0.195	0.298	0.048
Both seasons	0.818	0.077	0.864	0.070	0.575	0.156	0.440	0.114

Table 4. Daily fluoride intake from diet, toothpaste ingestion* and both sources, expressed as mg/d and mg/kg body weight per d, in children from four different areas of Iran
(Mean values and standard deviations)

Fluoride intake	Area							
	Shiraz (n 32)		Darab (n 28)		Dehkhir (n 10)		Hassan-Abad (n 8)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diet								
mg/d	0.318	0.077	0.364	0.070	0.575	0.156	0.440	0.114
mg/kg body weight per d	0.023	0.005	0.026	0.004	0.039	0.011	0.029	0.008
Toothpaste								
mg/d	0.104	0.073	0.011	0.033	0		0	
mg/kg body weight per d	0.007	0.005	0.0008	0.002	0		0	
Diet and toothpaste								
mg/d	0.422	0.126	0.374	0.065	0.575	0.156	0.440	0.114
mg/kg body weight per d	0.030	0.009	0.028	0.005	0.039	0.011	0.029	0.008

* The numbers of children who used a fluoride toothpaste were: Shiraz 30, Darab 3, Dehkhir 0, Hassan-Abad 0.

Table 5. Volume of urine excreted in 24 h, urinary fluoride concentration and fluoride excretion during summer and winter for children in four different areas of Iran
(Mean values and standard deviations for seventy-eight children)

Variable	Summer		Winter		Both seasons	
	Mean	SD	Mean	SD	Mean	SD
Volume of 24 h urine (litres)	0.457	0.214	0.474	0.213	0.465	0.172
Fluoride concentration of 24 h urine (mg/l)	0.770	0.358	0.690	0.295	0.730	0.263
Daily urinary fluoride excretion (mg)	0.352	0.116	0.327	0.126	0.339	0.100

992 g/g, while the corresponding range for the eight adult toothpastes was from 957 to 1219 g/g.

Main study

The parents of 116 (89%) of the 130 children invited to participate consented to the inclusion of their children in the study; seventy-eight children (67% of those who volunteered) completed all aspects of the study (Table 2). The mean heights of the boys and girls were 0.993 (SD 0.0588) m and 1.001 (SD 0.064) m respectively. The mean weight of the boys was 14.4 (SD 1.5) kg and it was 14.2 (SD 2.5) kg for the girls. The mean BMI was 14.7 (SD 1.58) for boys and 14.2 (SD 1.84) for girls. There was little difference in energy intake between boys (5.63 MJ/d) and girls (5.46 MJ/d) and the mean energy intake for both sexes combined was 5.56 (SD 1.06) MJ/d. These energy intakes were higher than standards for British children (Gregory *et al.* 1995) but the PAL value of 1.7 for these Iranian children was higher than the value of 1.4 for British children (Gregory *et al.* 1995) indicating a more active lifestyle for Iranian children and that under-recording of dietary intake was unlikely. For all children, the percentages of food energy derived from protein, fat and carbohydrate were 10, 29 and 61% respectively.

Of those who completed all aspects of the study, only thirty-three children (thirty children in Shiraz and three children in Darab) used a fluoridated toothpaste. Thirty of these thirty-three children agreed to participate in and completed the study investigating fluoride ingestion through toothbrushing. The mean quantity of fluoride ingested

during brushing was 0.109 (SD 0.125) mg in summer and 0.095 (SD 0.052) mg in winter, with an average of 0.102 (SD 0.073) mg fluoride for both seasons. As most of the children brushed their teeth just once daily, the mean daily fluoride intake from toothpaste (0.104 mg) for those who brushed with a fluoride toothpaste was almost equal to average fluoride ingestion per brushing episode.

Table 3 shows the fluoride intake from diet for all children in different areas. Despite similarity of the fluoride content of drinking water in these areas, fluoride intake tended to be higher in the rural areas than in the urban areas. Consumption of fluoride from diet was higher in summer in all areas.

The mean daily fluoride intakes of the seventy-eight children from diet, toothpaste ingestion and both sources, in the four areas, given as mg F and on a body-weight basis, are presented in Table 4. As none of the 4-year-old children in the rural areas and few of children in Darab used a fluoride-containing dentifrice, the total daily fluoride intake in these children was equal or nearly equal to the dietary intake. Of the total fluoride intake of the children in Shiraz 25% came from toothpaste ingestion.

The mean values of 24 h urinary creatinine excretion for these children were 17.9 and 18.6 mg/kg per d in summer and winter respectively. This is within the accepted range of 14–20 mg/kg per d (Harms & Scharfe, 1997) and indicates satisfactory urine collection. Table 5 summarizes the information on fluoride excretion in urine. About half of the children had a 24 h urine volume between 300 and 499 ml/d. A slightly lower mean 24 h urine output was observed with

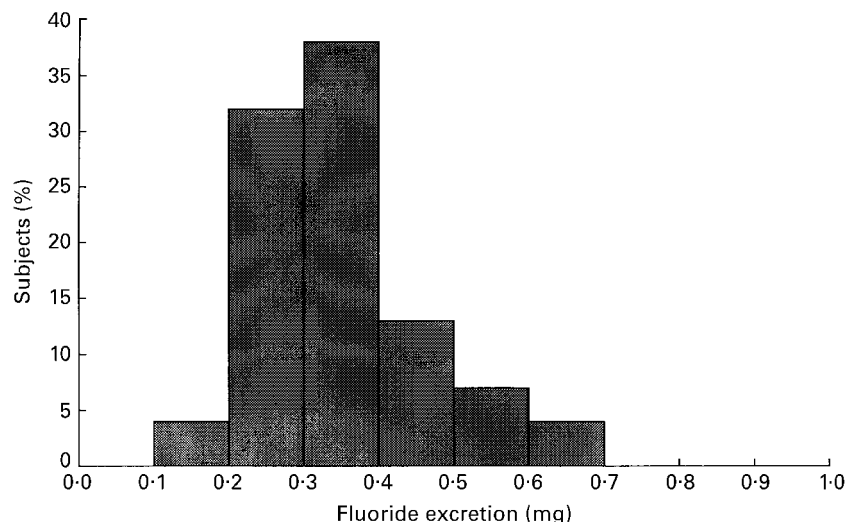


Fig. 1. Percentage frequency distribution of seventy-eight children according to weight of fluoride excreted (mg) in 24 h urine collections in areas of Iran with an average of 0.35 mg F/l in drinking water.

the higher environmental temperature of summer, while the mean urinary fluoride concentration was higher in summer than in winter. The mean weights of fluoride excreted in the two seasons were similar.

Fig. 1 illustrates the percentage frequency distribution according to weight of fluoride (mg/d) excreted in urine. A high proportion of children (fifty-five out of seventy-eight, 71%) excreted between 0.20 and 0.39 mg fluoride/d in urine. A similar frequency distribution is given in Fig. 2 for weight of fluoride ingested from all sources. Most of the children (49%) ingested between 0.30 and 0.39 mg F/d. A positive ($P = 0.005$) correlation was found between fluoride intake and urinary fluoride excretion (Fig. 3). The slope of the best fit line (+0.25) was different from the solid line in the figure which represents, hypothetically, 100% excretion

of ingested fluoride. The 95% CI for the slope was +0.054 to +0.443.

Table 6 presents fluoride intake and urinary fluoride excretion for the seventy-eight children in the four areas. Averaged over all children and both seasons, the difference (fluoride intake minus urinary fluoride excretion) was +0.087 (SD 0.443) mg/d, with some variation between the four areas. This represents 80% excretion of ingested fluoride.

Discussion

This is the first report of fluoride intake and urinary fluoride excretion in children over 2 years of age, except for one published abstract (Brunetti & Newbrun, 1983). It shows

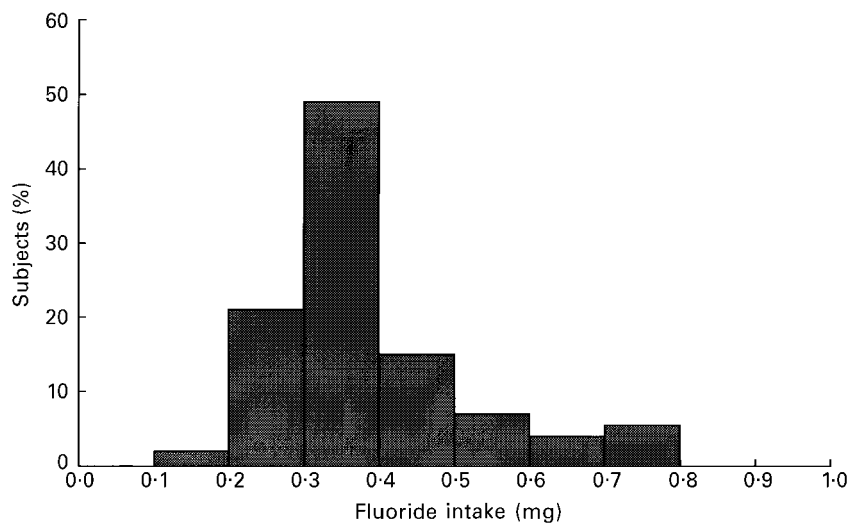


Fig. 2. Percentage frequency distribution of seventy-eight children according to weight of fluoride ingested (mg/d) in areas of Iran with an average of 0.35 mg F/l in drinking water.

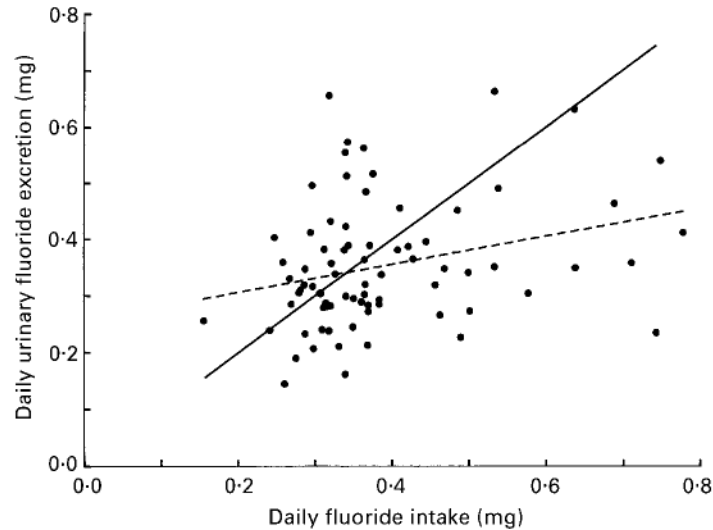


Fig. 3. Relationship between total fluoride intake and urinary fluoride excretion for seventy-eight 4-year-old children in Iran. (----), Best fit straight line for that relationship; (—), hypothetical 1 : 1 ratio. The relationship can be expressed as: amount of fluoride excreted (mg) = $0.25 + 0.25$ fluoride intake (mg), ($P < 0.005$, $r^2 = 0.08$).

Table 6. Total fluoride intake (mg), urinary fluoride excretion (mg) and the difference (mg) per d for seventy-eight children in four areas of Iran, averaged over summer and winter seasons (Mean values and standard deviations)

Area	n	Fluoride intake (mg)		Fluoride excretion (mg)		(Intake - excretion) (mg)	
		Mean	SD	Mean	SD	Mean	SD
Urban:							
Shiraz	32	0.422	0.426	0.803	0.080	+0.419	0.436
Darab	28	0.874	0.065	0.878	0.115	+0.004	0.659
Rural:							
Dehkhir	10	0.575	0.456	0.441	0.104	+0.134	0.461
Hassan-Abad	8	0.440	0.114	0.848	0.139	+0.092	0.008
Total	78	0.426	0.426	0.839	0.100	+0.087	0.443

that, contrary to current opinion (World Health Organization, 1986), urinary fluoride excretion was, on average, 80 % of fluoride intake. Children aged 4 years were the subjects of the present study, as most permanent teeth are forming at this age and it is therefore a pertinent age to investigate fluoride retention; dietary and toothbrushing habits are becoming established and children are usually able to control their urination and cooperate in this type of study.

All aspects of the study were undertaken in summer and winter in order to assess any seasonal changes, as water is a major source of fluoride intake, and to estimate the average fluoride intake and excretion. In addition, a higher reliability of the estimates of dietary intake was likely in repeated short surveys compared with the same number of days surveyed in a single period (Hackett *et al.* 1983). The repeated 3 d dietary diary method with interview before and after completing the diaries provides a good estimate of the mean intake and distribution of intake for groups (Adamson *et al.* 1992). When expressed on a body-weight basis, the mean

energy intake of these children was in broad agreement with UK and WHO recommendations (Department of Health, 1991). Bingham (1994) reported that the ratio energy intake : BMR (PAL) is a suitable and simple check on the validity of group estimates of energy intake. The PAL ratios of 1.7 for both sexes in the present study are compatible with standards (1.8) but higher than that reported for British children (1.4) (Gregory *et al.* 1995). Therefore, the method of collecting dietary information in the present study appeared to be valid, at least for food energy. In order to prevent any underestimation of tap water intake (as a major source of fluoride intake but without a contribution to energy intake), parents were asked to use a jar or bottle full of water at the beginning of the study and measure the amount of water left inside at the end of each day. The importance of recording in the diary the number of glasses or cups of all drinks consumed away from home was strongly emphasized. While the recorded water intake of 1136 g/d in this study was less than the theoretical

suggestion of McClure (1943) (1600 g/d for children aged 4–6 years), the total water intake per kg body weight of these Iranian children was very similar to that recorded for American children (Ershaw & Cantor, 1989): 81 and 78 g/kg body weight per d for Iranian and American children respectively.

The study was undertaken in areas receiving drinking water with low to moderate levels (0.3 mg/l) of fluoride, as established in a preliminary survey (Table 1). The three localities were chosen to provide some contrast in lifestyle and therefore in dietary habits. Within these localities the kindergartens or health centres were chosen randomly. Of those sampled, 89% agreed to participate and the completion rate was 67% of volunteers. Because of the nature of the study, which demanded the cooperation of parents for their close recording of their child's diet over 3 d, collection of urine over 24 h and a toothbrushing exercise in their home, in summer and winter, the completion rate obtained was acceptable.

For all children, averaged over all 6 d, the mean intake of fluoride from the diet was 0.39 mg/d. There was variation between the four localities (Table 3) and further analyses have shown that this was because of a greater consumption of tea in the rural areas. There is no report on the dietary fluoride intake of children aged 4 years in an area receiving a similar amount of fluoride in drinking water as in this study. However, Schamschula *et al.* (1988b) reported mean dietary fluoride intakes of 0.22 and 0.72 mg/d for Hungarian children aged 3–4 years residing in areas with water fluoride concentrations of less than 0.1 mg/l, and 0.5–1.4 mg/l respectively. Guha-Chowdhury *et al.* (1996) found the average dietary fluoride intake of New Zealand children aged 4 years living in a low-fluoride area, to be 0.45 mg/d which was considerably less than that found in the present study (0.32–0.58 mg F/d). As the technique of fluoride analysis was the same in these studies (the present study and those of Schamschula *et al.* 1988b and Guha-Chowdhury *et al.* 1996), differences in results are probably due to differences in the fluoride content of water and foods in different areas, and variation in quantities consumed.

As no fluoride supplements were taken by these children the total daily fluoride intake was provided by diet and dentifrice ingestion. The diet was the only relevant source of fluoride intake in children residing in rural areas in the present study. The total daily fluoride intake in Shiraz, where most of the children brushed their teeth, was estimated to be 0.42 mg, of which 25% (0.10 mg/d) came from toothpaste ingestion (Table 4). In comparison, the contribution of toothpaste ingestion to total daily fluoride intake (excluding fluoride supplements) in a low-fluoride area of New Zealand has been reported to be 0.34 mg/d or 69% of the mean daily total intake of 0.49 mg, for children aged 3–4 years (Guha-Chowdhury *et al.* 1996). Thus, although the mean total daily intakes were rather similar, the amounts coming from diet and toothpaste in these two groups of children were rather different.

On a body-weight basis, the mean total fluoride intake of Iranian children was 0.030 mg/kg body weight per d, which is almost equal to that (0.027 mg/kg body weight per d) reported by Guha-Chowdhury *et al.* (1996) for 3–4-year-old New Zealand children living in low-fluoride areas with

0.2–0.3 mg F/l in public water supplies. The average daily diet of American children aged 1–12 years, based on body weight, was estimated by McClure (1943) to contain 0.05–0.07 mg F/kg body weight per d, which has been interpreted as an 'optimum level' by some investigators (Ophaug *et al.* 1985), or an 'upper limit' of fluoride intake from all sources by Burt (1992). Since, on average, the mean fluoride ingested from toothbrushing was 0.007 mg/kg body weight per brushing episode for each child who brushed, if a child brushes his or her teeth three times per day, the total fluoride intake from diet and toothpaste ingestion will reach the optimum level. Use of fluoride dietary supplements would not be recommended for these children.

The cooperation of the volunteers in collecting urine for 24 h was very good. A few children who had night urination repeated the collection, sometimes even three times, in order to obtain a total 24 h urine sample. Urinary creatinine excretions for these children were within the standard range, confirming the accuracy of 24 h urine collection. The lower 24 h urinary volume recorded in summer (Table 5) is in good agreement with previous reports on a lower volume of 24 h urine with higher environmental temperatures. Crosby & Shepherd (1957) reported 24 h urine outputs of 537 and 485 ml in winter and summer respectively, for Australian children aged 3–5 years, which are slightly higher than the corresponding values for Iranian children (490 ml in winter and 443 ml in summer). Mean 24 h urine volumes of 504 and 449 ml for children aged 4 years living in Sri Lanka and England respectively, were reported by Rugg-Gunn *et al.* (1993), and for Jamaican children aged 2–6 years old, 302 and 461 ml in two areas (Warpeha & Marthaler, 1995). The distribution of urine volume in the present study is quite similar to those reported by Rugg-Gunn *et al.* (1993) and Crosby & Shepherd (1957). Since changes in the source of drinking water could affect the retention of fluoride in the body towards temporary positive or negative balances, any child who had not lived permanently inside their current area of residence for at least 3 years, was excluded from the study.

There are few studies in which the total weight of fluoride excreted in 24 h urine has been measured, partly due to the difficulties of obtaining 24 h urine collections, especially in young children. Rugg-Gunn *et al.* (1993) calculated the mean weight of fluoride excreted in 24 h urine samples of 4-year-old children consuming drinking water containing close to 1.0 mg F/l in England and Sri Lanka, and reported values of 0.55 and 0.42 mg in Sri Lanka (*n* 53) and England (*n* 44) respectively. These values are higher than that obtained for Iranian children (0.34 mg/d) (Table 5) probably because of the higher water fluoride concentrations. However, the mean daily fluoride excretion in urine (0.33 mg) which was reported recently (as an abstract) for 5–6-year-old Chinese children residing in areas with 0.3 mg F/l in the water supply (Wang *et al.* 1997 and personal communication), is almost equal to that recorded in the present study.

Slightly less than 20% of ingested fluoride was retained in the body, in strong contrast to the assumption that at least 70% is retained by pre-school children (World Health Organization, 1986). In a fluoride balance study on eleven infants (Ekstrand *et al.* 1994), the mean fluoride retention was reported to be 12.5 (SD 13.8)% of intake, with a range

from -13.5% to $+33.2\%$ when the only source of fluoride intake was the diet. This study involved measurement of total fluoride intake and fluoride excretion in urine and faeces. The situation regarding fluoride intake was fairly similar to the present study, where the contribution of fluoride toothpaste to fluoride intake was small. The mean fluoride intake in the study of Ekstrand *et al.* (1994) was $20.5 \mu\text{g/kg}$ per d, and $30 \mu\text{g/kg}$ per d in the present study. The only comparable result for children over 2 years is an abstract by Brunetti & Newbrun (1983) who recorded a mean fluoride intake of 0.83 mg/d and fluoride excretion of 0.28 mg/d with a difference of $+0.05 \text{ mg/d}$ and retention of 15% , in ten 3–4-year-old children living in a fluoridated community in California. This compares with the estimated fluoride 'retention' of $+0.087 \text{ mg/d}$ or 20% in the present study (Table 6). As no faeces samples were collected in this study (but were in the study of Brunetti & Newbrun, 1983), actual fluoride retention and fluoride balance (fluoride intake \cdot fluoride output from urine and faeces) cannot be calculated directly in our study. Faecal fluoride usually accounts for less than 10% of ingested fluoride (Ekstrand, 1996). Assuming 10% of the fluoride intake in the present study was excreted through faeces, the total fluoride retention through urine and faeces would be 0.881 mg/d : the fluoride balance and retention in these Iranian children would then become $+0.05 \text{ mg}$ and 11% respectively, which is in a good agreement with the study of Brunetti & Newbrun (1983). A lower positive fluoride balance has also been recorded for subjects who had consumed drinking water containing 0.5 mg F/l for years (Machle *et al.* 1942; Largent, 1961). Negative balances in other fluoride balance studies in different age groups have been reported, with fluoride intakes of less than 0.5 mg/d (Maheshwari *et al.* 1981a,b) and breast-fed infants (Ekstrand *et al.* 1984). On the other hand, positive balances have always been found in studies of subjects ingesting doses of the magnitude of $5\text{--}10 \text{ mg F/d}$ for relatively short periods (Spencer *et al.* 1970; Maheshwari *et al.* 1981b) and in formula-fed infants (Ekstrand *et al.* 1984).

The low retention of fluoride in Iranian children might be a feature of their vegetarian diet, as such a diet would lead to more alkaline urine which is associated with greater fluoride excretion (Whitford, 1990). Further epidemiological studies are required to investigate the importance of this factor. These Iranian children consumed more carbohydrate and less fat than British children: the amount of protein consumed was little different although only 30% of protein came from animal sources and the majority came from legumes and cereals in the Iranian children. Their diet could, therefore, be described as a high-carbohydrate diet based on vegetable sources. Lunch is usually the main meal and is usually eaten at home.

It would appear from limited literature that fluoride excretion via sweat is negligible, even in active children in a hot climate. Using published data (Henschler *et al.* 1975), Ekstrand (1996) calculated that in tropical climates, during periods of prolonged or heavy exercise, or in occupational exposure to elevated temperatures, where fluid loss via sweat might be as much as $4\text{--}6 \text{ litres/d}$, the excretion of fluoride via sweat would not exceed 0.4 mg/d .

It should be noted that a minimum retention of dietary Ca

in children under 10 years has been observed to occur at the age of 4 years (Kanis & Passmore, 1989). Retention of Ca in children aged 4 years is about 10% of intake compared with about 40% at 0.5 year, 25% at 1 year, 15% at 2 years and 80% at 10 years. The lower retention of Ca in 4-year-olds coincides with a lower growth rate for this age-group of children, compared with other age groups up to 10 years. As retention of fluoride depends on the stage of skeletal development, lower fluoride retention might also occur for children aged 4 years (in parallel with low Ca retention). According to a review on Ca balance by Matkovic & Heaney (1992), Ca balance can be calculated using the following formula for children 2–8 years old: $\text{Ca balance (mg/d)} = 0.43 \times 55 + (0.238) \text{ Ca intake (mg/d)}$. Using this formula, the Ca retention was calculated to be 12% for these Iranian children based on Ca intake of 388 mg/d recorded in this study. This value of 12% retention of Ca at age 4 years is fairly similar to our value for fluoride retention at the same age: 20% without, or 11% with, allowance for faecal fluoride excretion. However, it must be appreciated that these values for Ca retention are theoretical calculations, but suggest that percentage fluoride (and Ca) retention may vary with age in childhood.

This study is the first to record in detail total fluoride intake and urinary fluoride excretion in children. The fact that the results do not agree with previous theoretical suggestions indicates a need for further research on fluoride balance in children of various ages consuming different types of diet.

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